METABONOMICS TECHNIQUES WITH APPLICATIONS TO PREDICTIVE TOXICOLOGY

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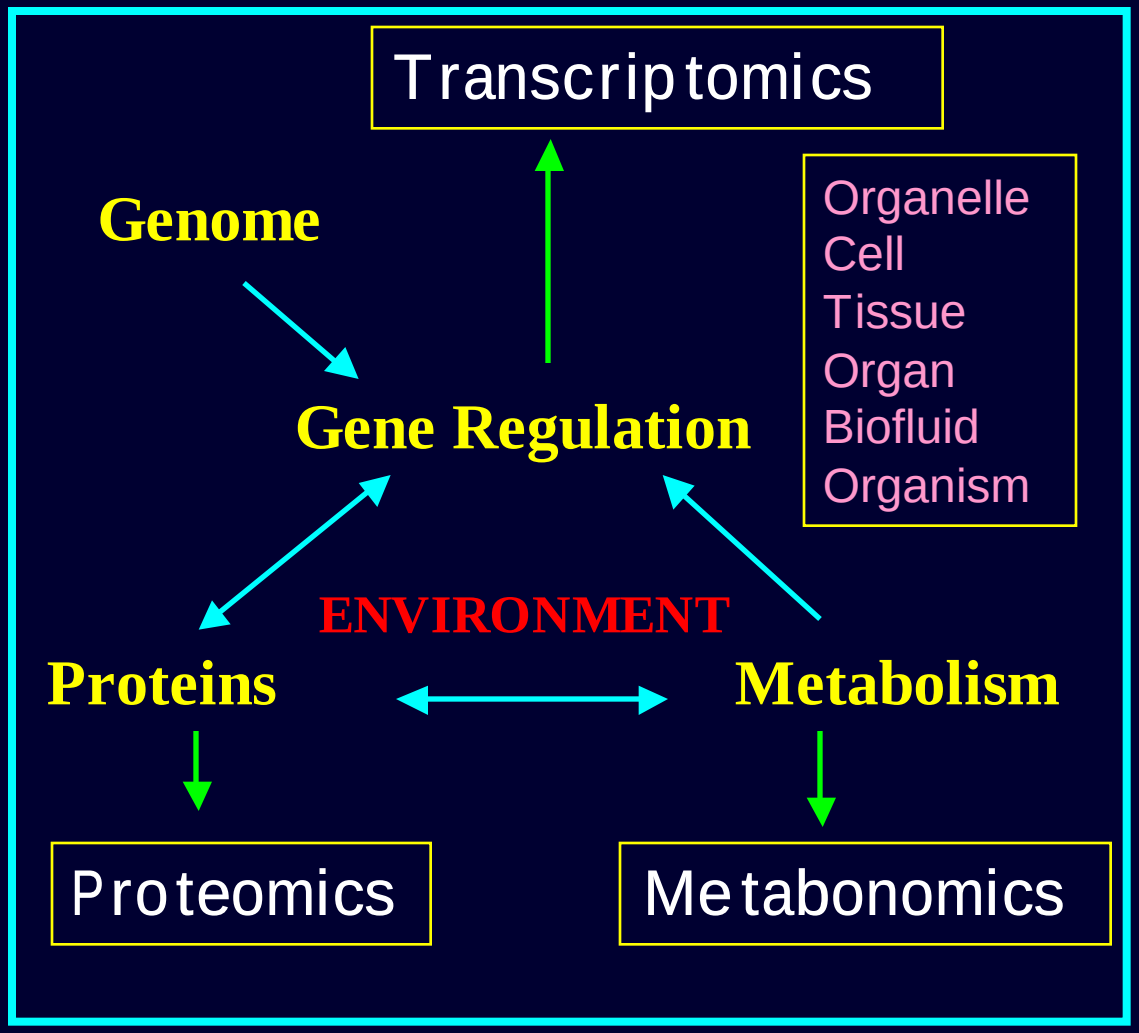
RESPONSES AT DIFFERENT LEVELS OF BIOMOLECULAR ORGANISATION



METABONOMICS is the quantitative measurement of time-related multiparametric metabolic responses of living systems to pathophysiological stimuli or genetic modification

Systems approach, includes biofluids and tissues

POSSIBLE ANALYTICAL APPROACHES FOR METABONOMICS

Conventional clinical biochemistry parameters

All types of spectroscopy - molecular information at several levels

- Chromophores
- Functional groups
- Atom-specific

Infra-red (IR) spectroscopy

Mass spectrometry (MS), including LC-MS

Nuclear magnetic resonance (NMR) spectroscopy

HIGH RESOLUTION NMR OF BIOFLUIDS AND TISSUES

- **Biofluids (standard liquid state NMR methods)**

  Plasma, Urine, Cerebrospinal

  Saliva, Gastric, Bile, Pancreatic

  Amniotic, Follicular, Milk, Seminal Vesicle, Prostatic, Seminal

  Ascites, Cystic, Blister

  Dialysis fluids, Lavage fluids, Aspirates

- **Intact Tissues (MAS NMR methods)**

  Liver, Kidney, Prostate, Brain, Gut, Blood, Skeletal and Heart Muscle, Lymphoid, Bone, Cartilage

METABONOMICS ANALYTICAL STRATEGIES

Biofluids and tissues

Extraction (and derivatization)

Intact

NMR

GC-MS

LC-MS^n LC-MS^n-NMR

ICPMS-TOFMS

^1H, ^{13}C, ^{31}P NMR

2D NMR

LC-NMR

Diffusion measurements

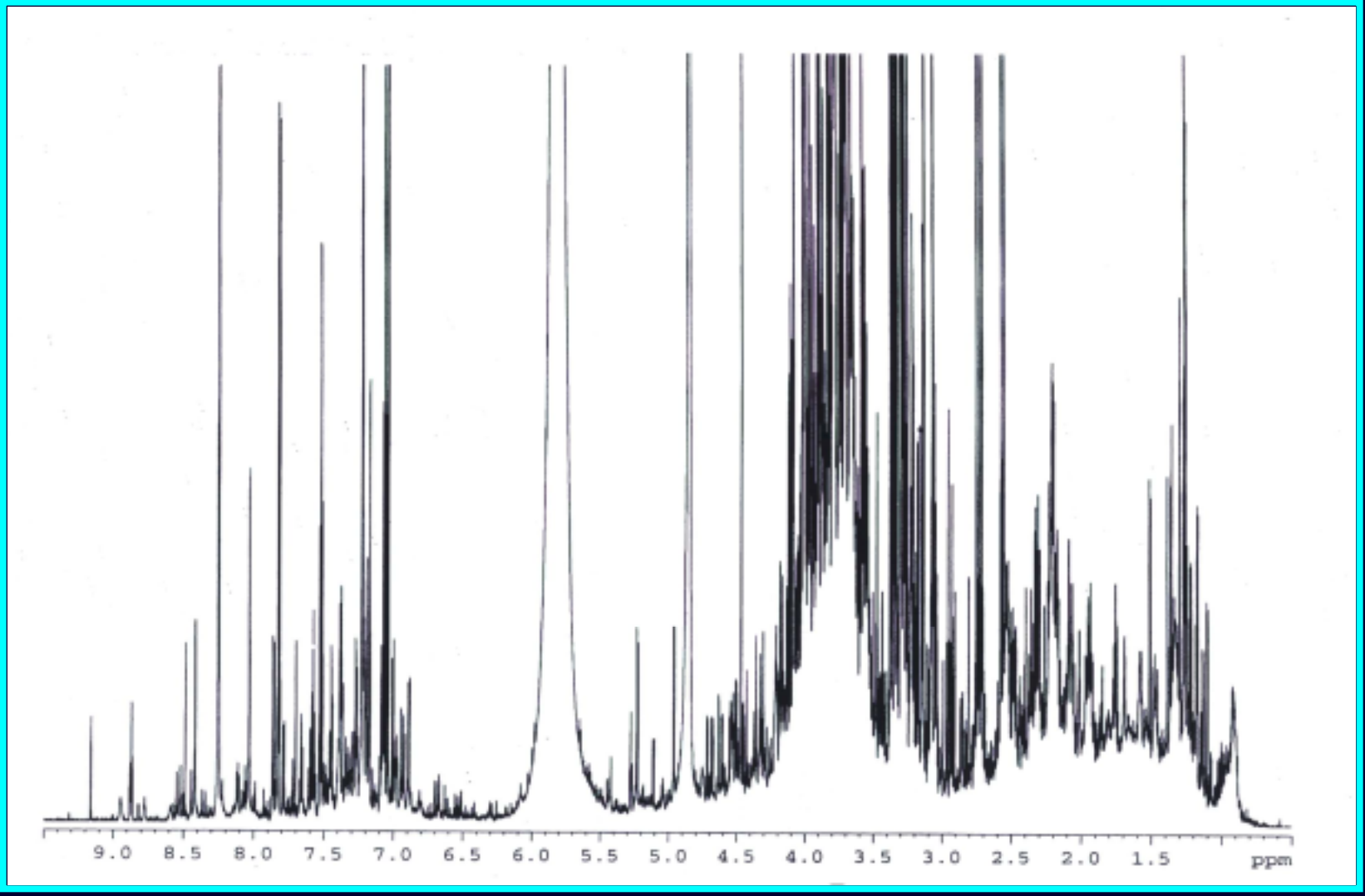
MAS-NMR

Identification of biomarkers, quantitation, ,molecular dynamics, interactions, compartmentation

ADVANTAGES OF ^{1}H NMR SPECTROSCOPY

- Non-destructive, generally non-invasive and low cost per sample
- Wide range of metabolites detectable, no pre-selection required
- Small sample volumes (typically 0.3-0.5 ml and as low as 3 μl)
- Little sample preparation and acquisition of NMR data is rapid (2-3 min)
- High level of structural information
- Observation of dynamic interactions of molecules

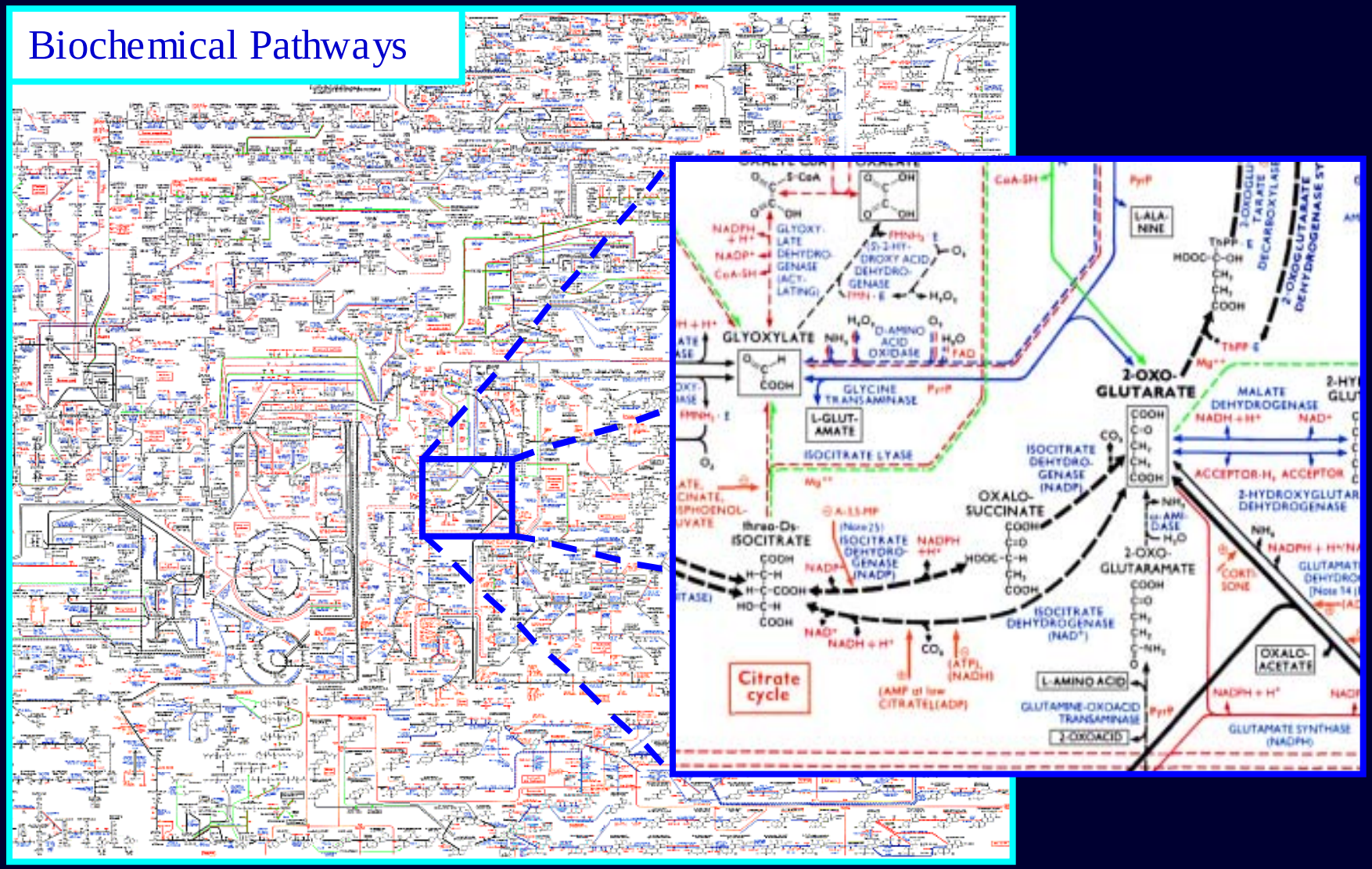
900 MHz ^{1}H NMR SPECTRUM OF HUMAN URINE



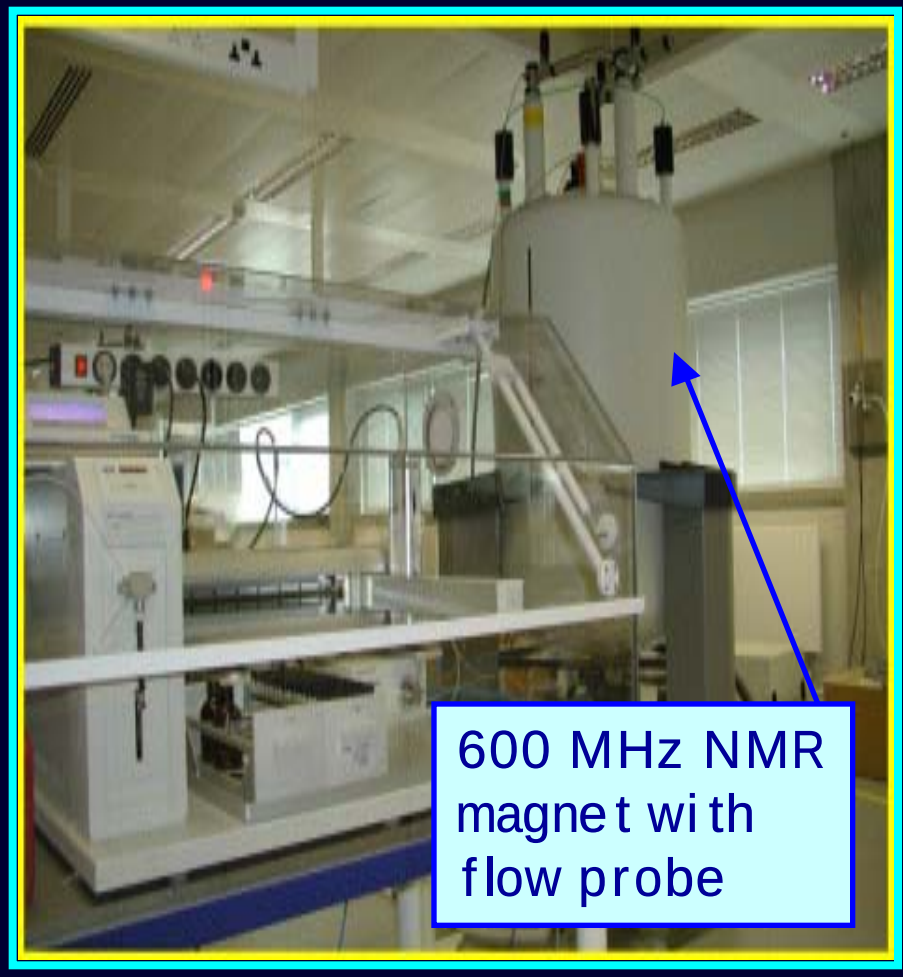
Intensity – relates to concentration and hydrogen count

Radiofrequency – relates to chemical identity

BIOCHEMICAL PATHWAYS



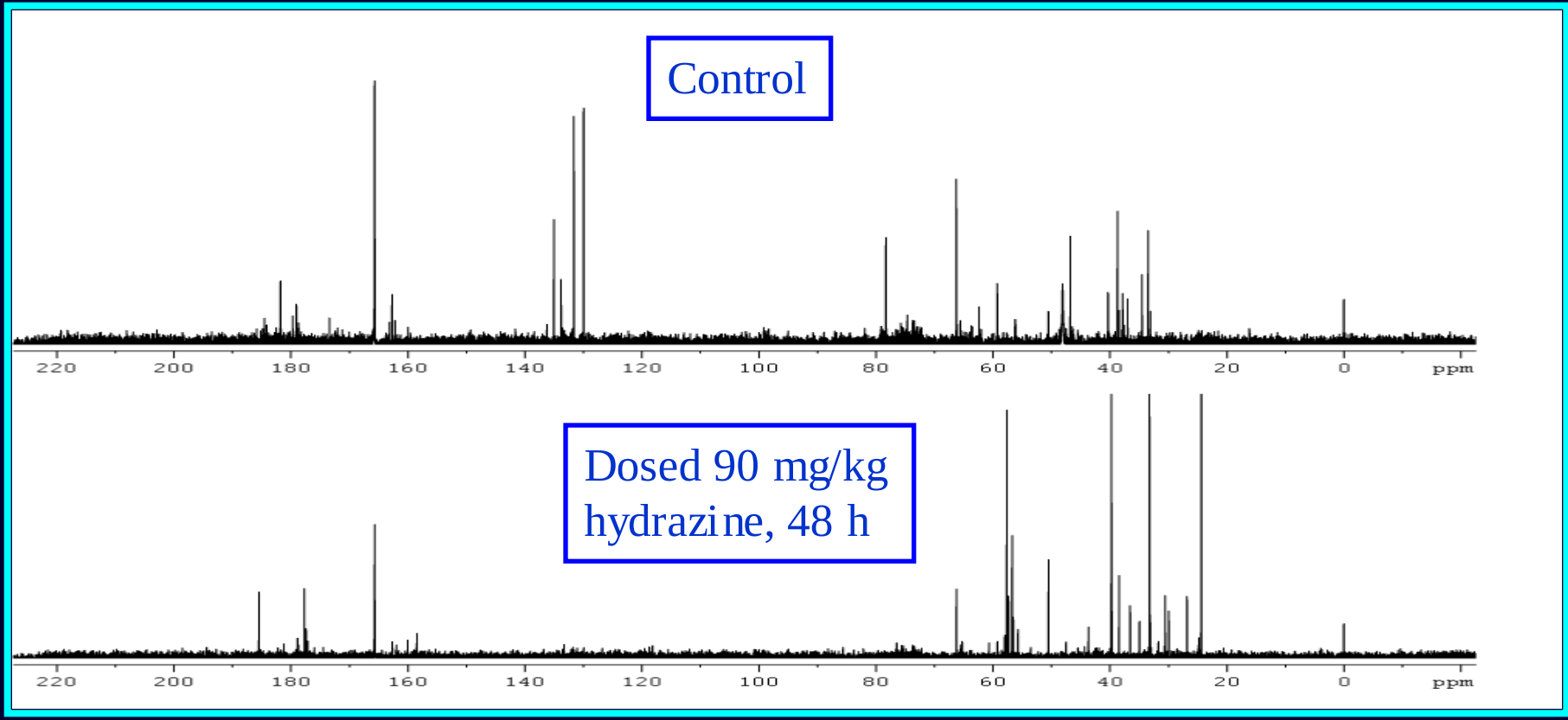
ROBOTIC SAMPLE PREPARATION AND MEASUREMENT



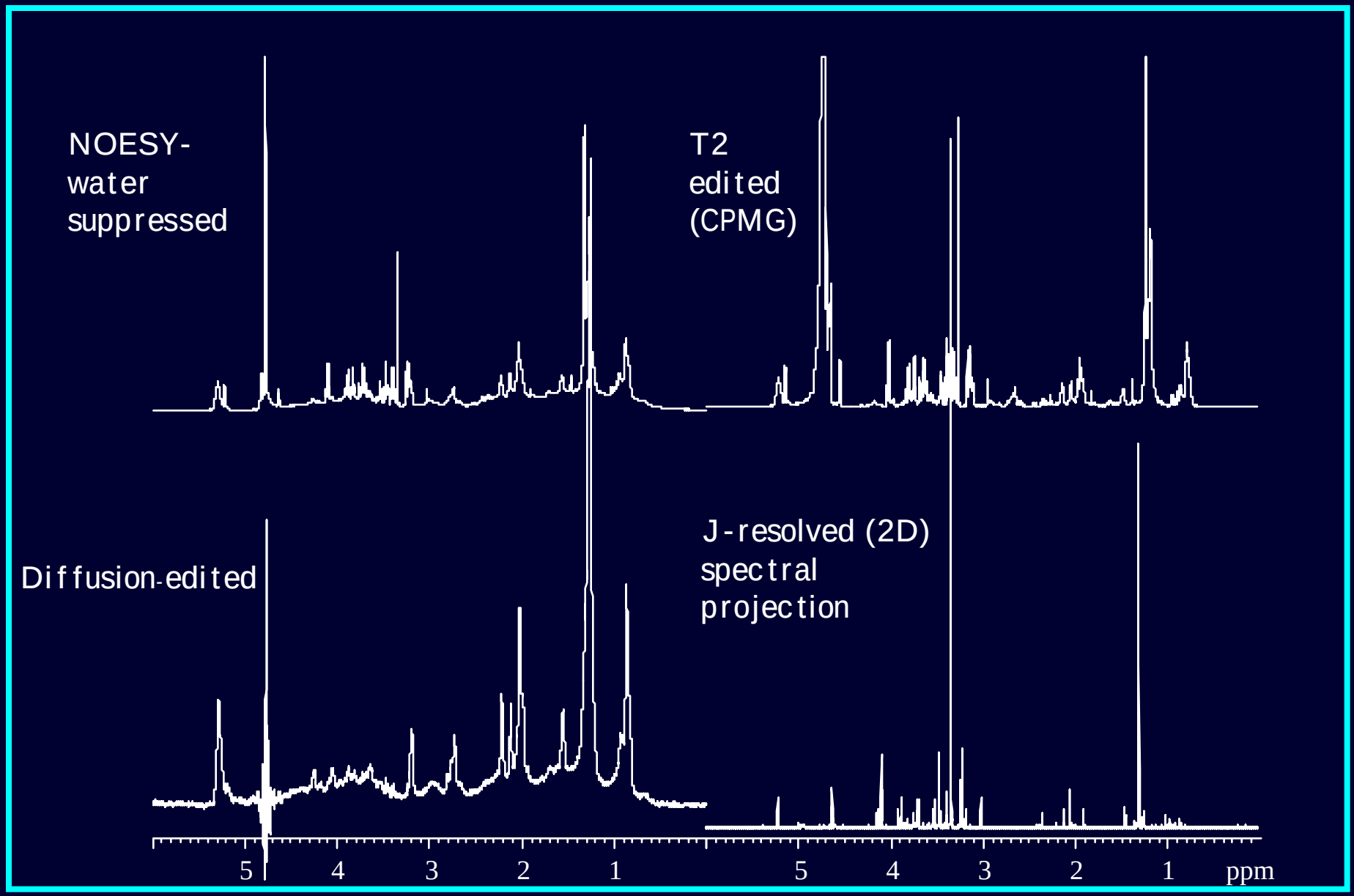
^{13}C CRYOPROBE NMR SPECTRA

500 MHz ^{13}C/^{1}H dual probe

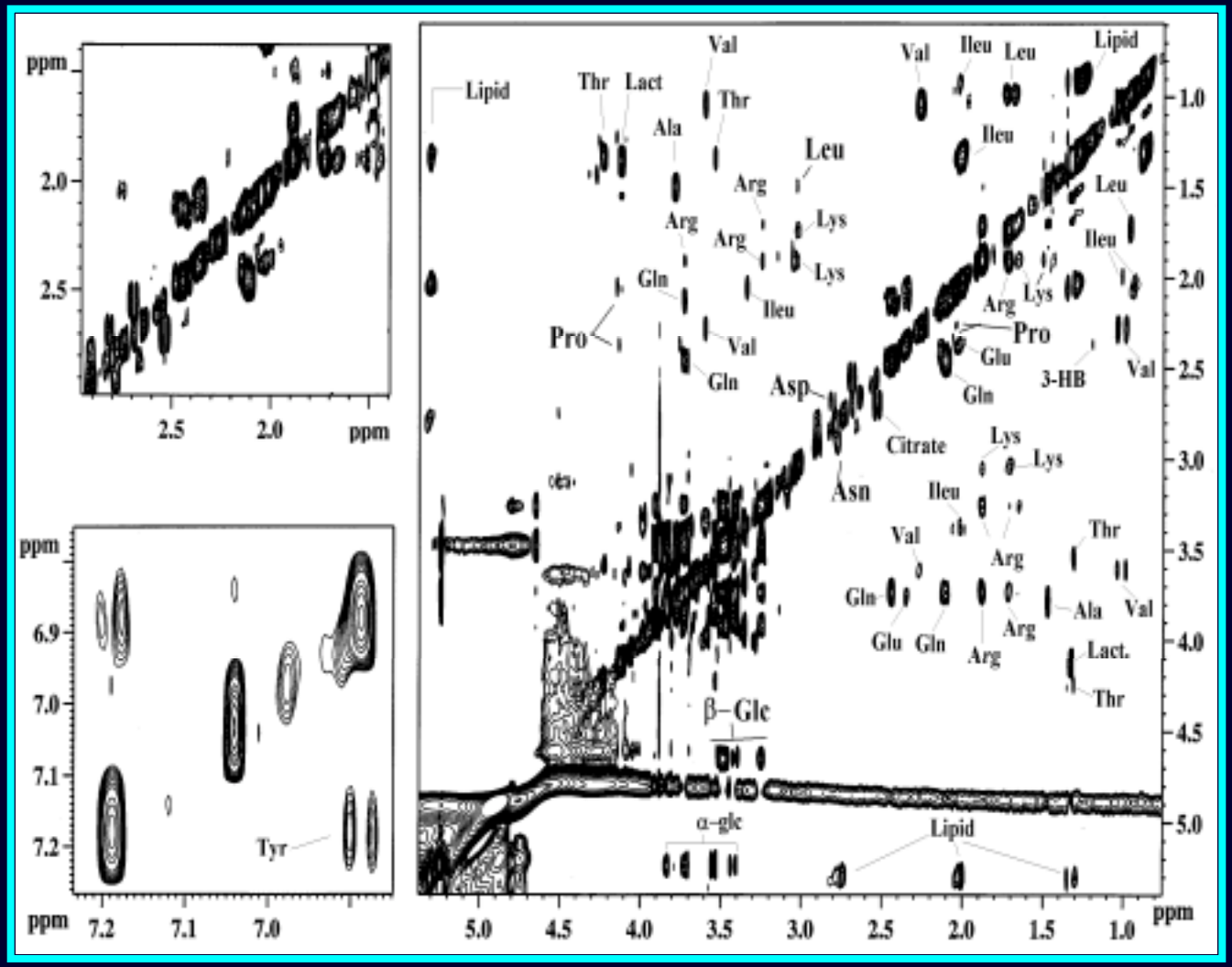
Rat urine diluted 2:1 with buffer, 512 scans, 30 minute acquisition



600 MHz NMR SPECTRA OF RAT SERUM



2-DIMENSIONAL NMR SPECTRA OF BIOFLUIDS

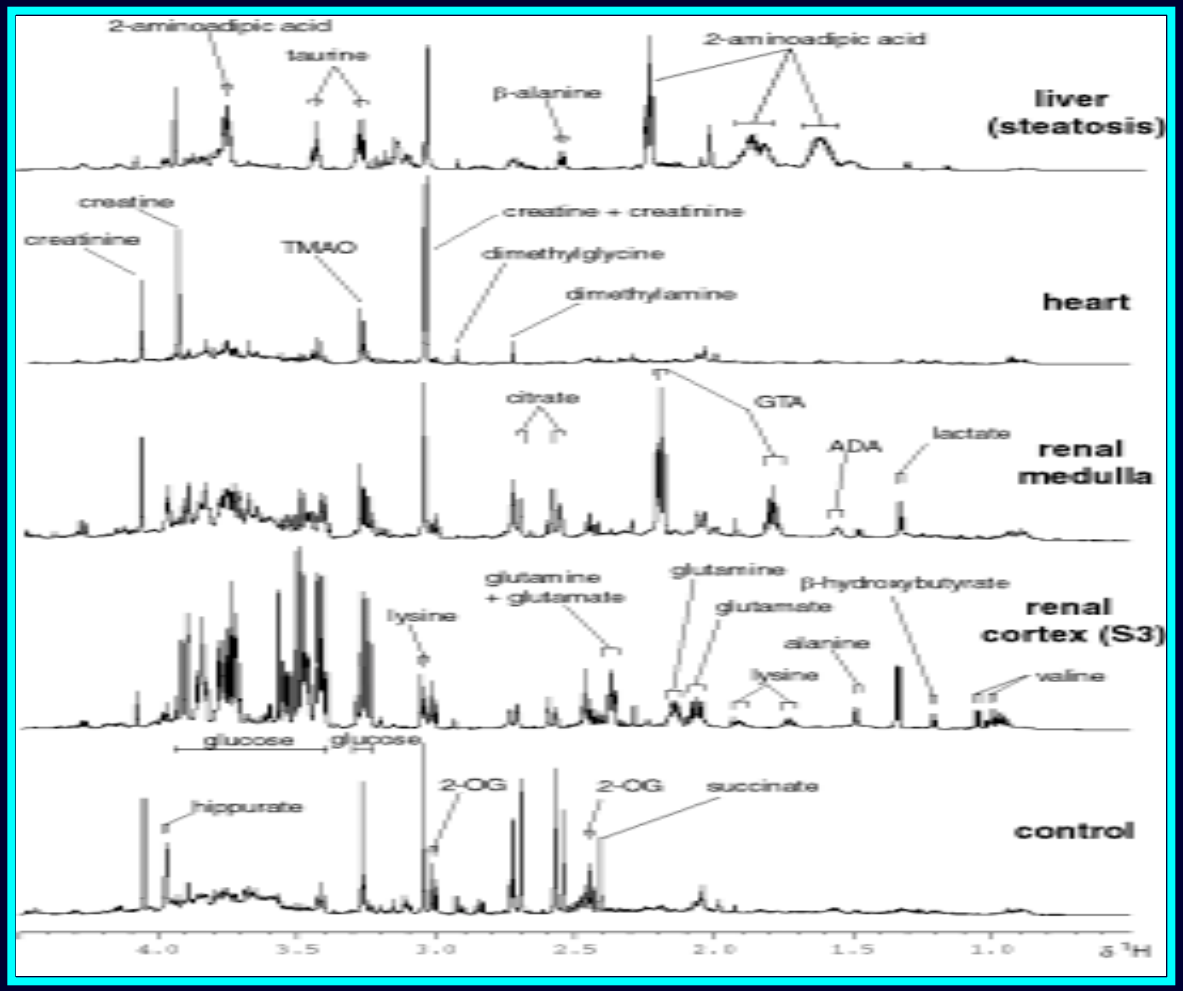


T_2-edited ^1H-^1H TOCSY NMR spectrum of human blood serum

Can also do heteronuclear-proton correlations -

^1H-^{13}C, ^1H-^{31}P

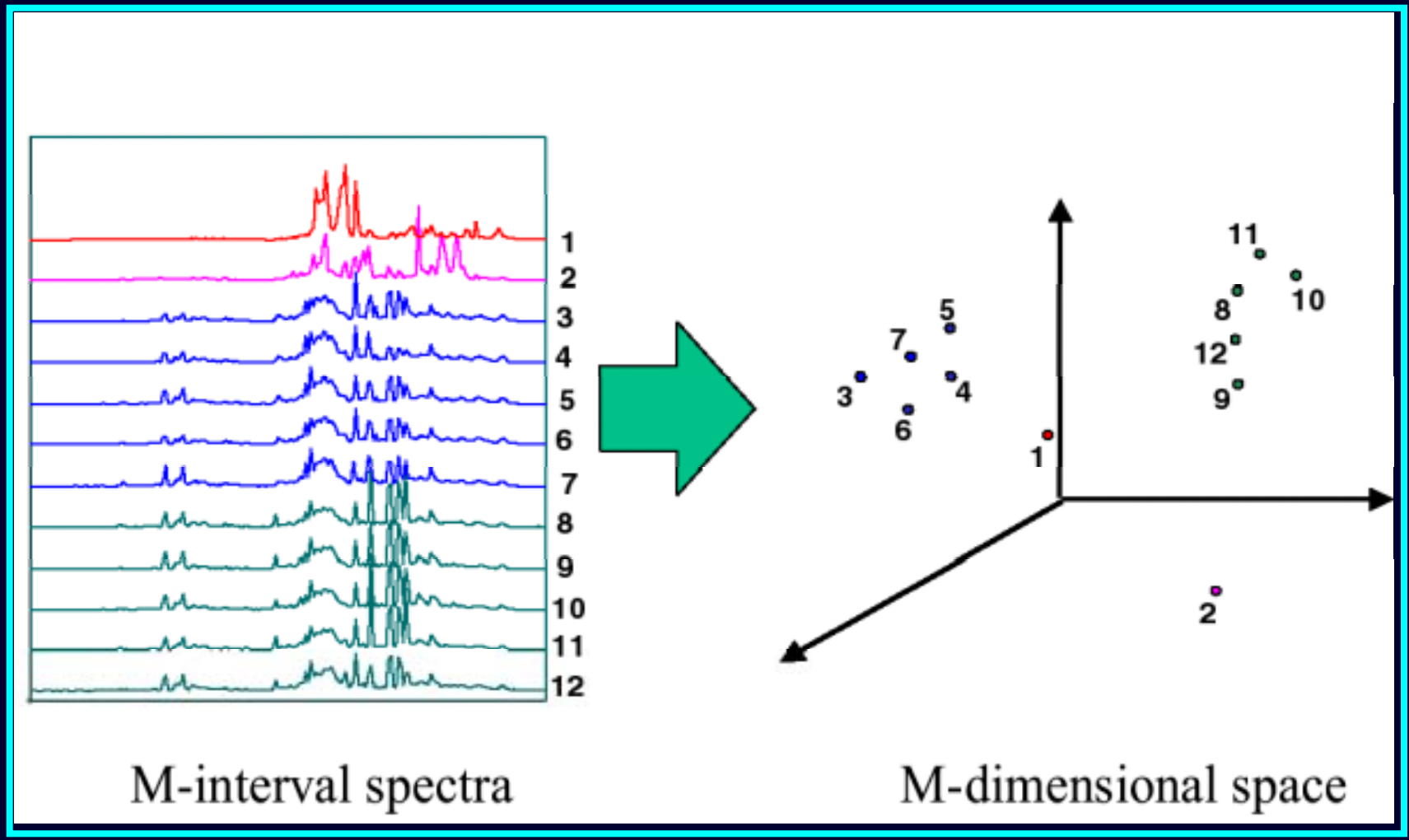
^{1}H NMR SPECTRA OF URINE SAMPLES FROM ANIMALS TREATED WITH TOXINS



600 MHz ^{1}H NMR spectra showing the effect of tissue-specific toxins on the metabolic profile of urine

Changes reflect the site and/or mechanism of toxicity

REPRESENTATION OF SPECTRA IN MULTIDIMENSIONAL SPACE



SOME PATTERN RECOGNITION AND EXPERT SYSTEM METHODS

UNSUPERVISED

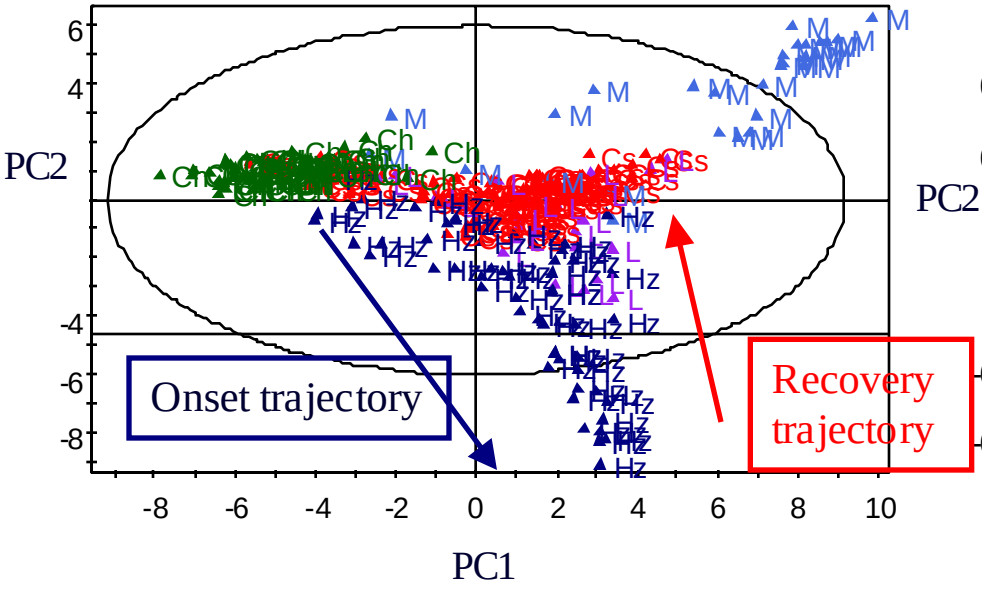
Non-linear Mapping
Principal Components Analysis
Hierarchical Cluster Analysis
Batch modelling
Etc.

SUPERVISED

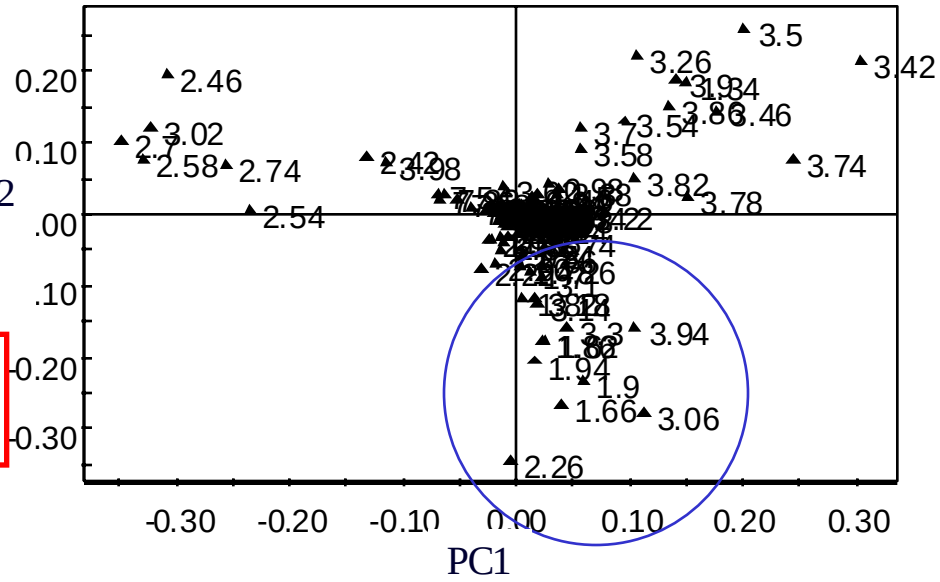
Partial Least Squares Discriminant Analysis
Rule Induction
Neural Networks
Soft Independent Modelling of Class Analogy (SIMCA)
Orthogonal Signal Correction
Etc.

SCORES AND LOADINGS IN PCA: NMR SPECTRA OF URINE

Scores plot shows sample clustering



Loadings plot shows NMR regions responsible for clustering



Each point is an NMR spectrum from a rat, either control (red) or dosed with 1 of 3 toxins (green, blue, purple), collected up to 7 days

Diagnostic spectral regions (metabolic descriptors)

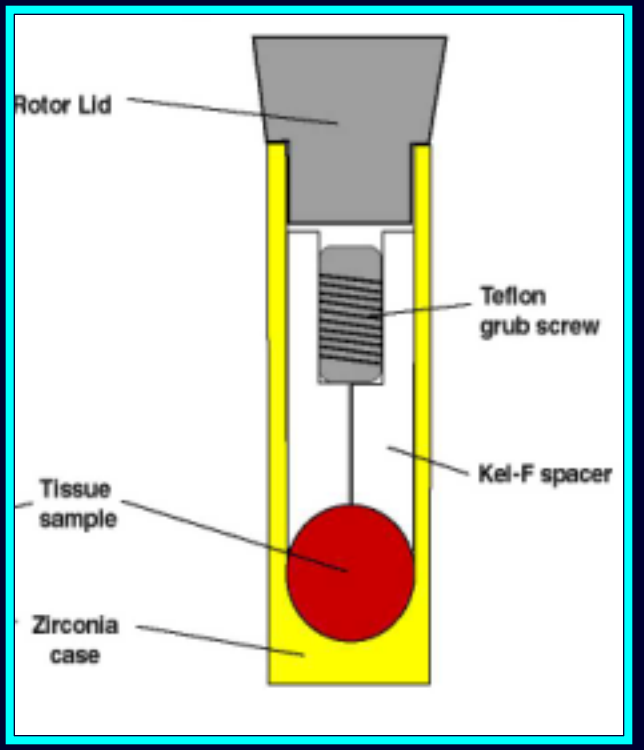
^{1}H NMR SPECTROSCOPY OF TISSUES

Uses a technique called magic-angle spinning (MAS) to remove effects which cause peak broadening resulting in loss of biochemical information

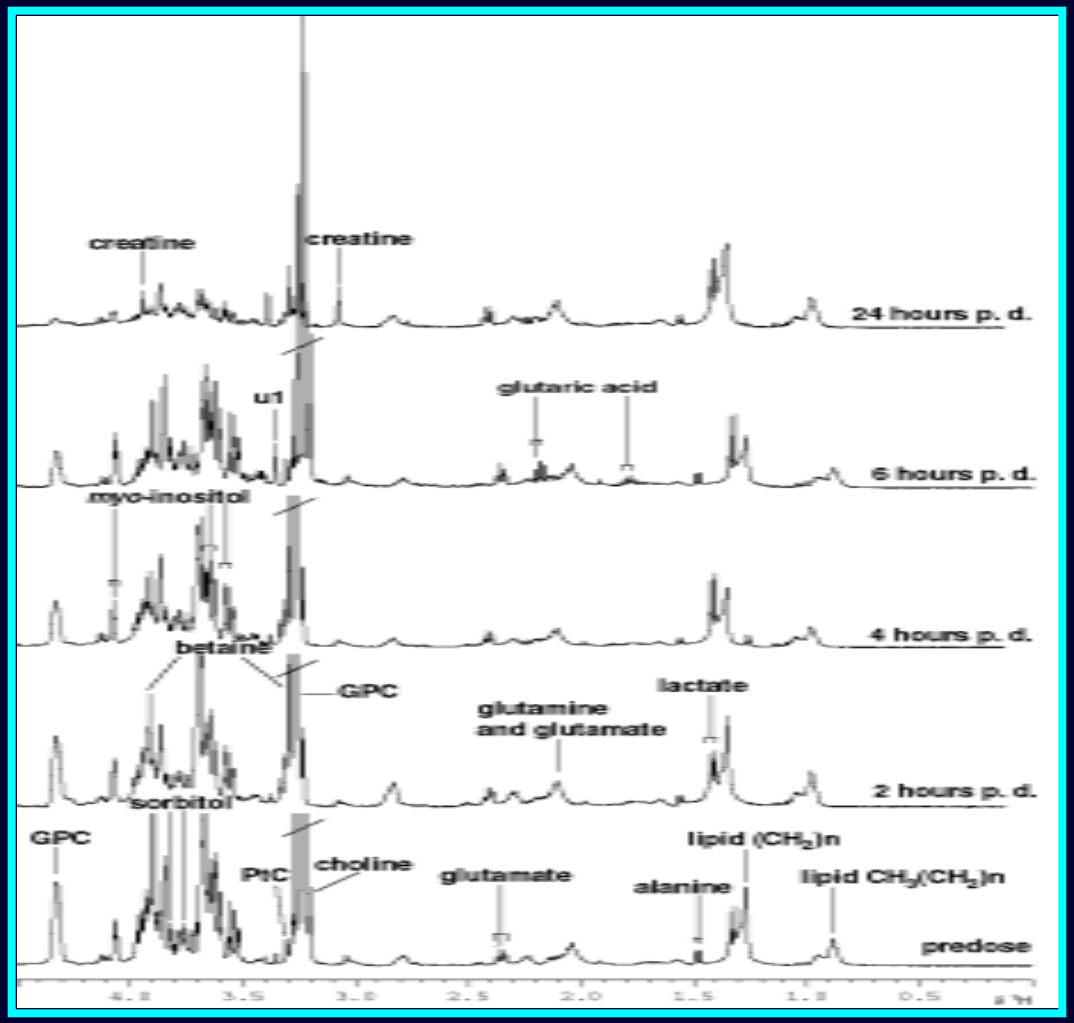
Tissues are not rigid solids, some molecular tumbling occurs, reducing the broadening – thus only modest spin rates required 250,000 – 750,000 r.p.m!

Can edit the NMR spectra to show only small molecular weight metabolites or macromolecules selectively

Can also probe molecular dynamics and compartmentation



^{1}H MAS NMR SPECTRA OF RAT RENAL PAPILLA TISSUE – TIME DEPENDENCE

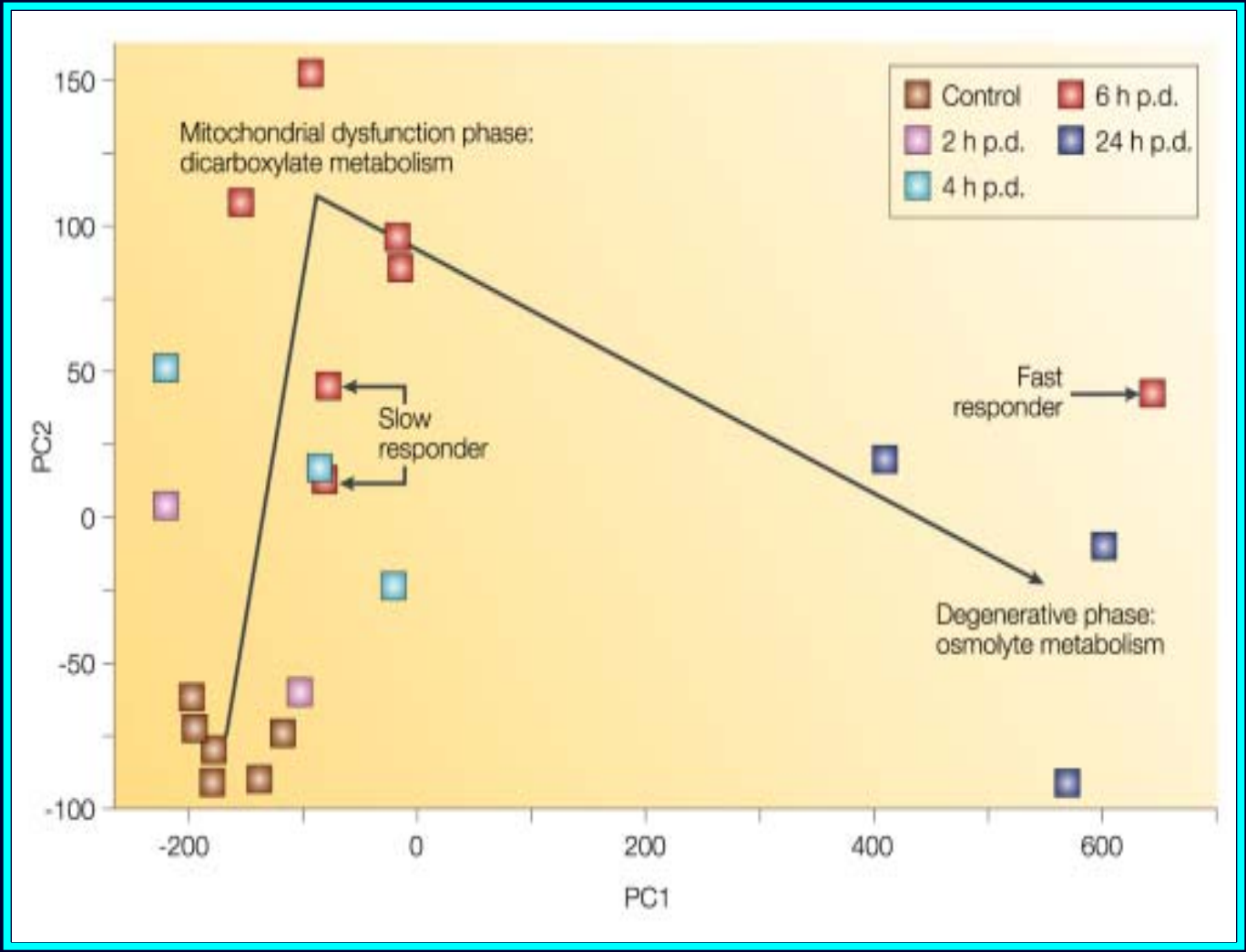


BEA is a model toxin used to study renal papillary necrosis.

Depletion in the levels of osmolytes in the kidney tissue indicates specific damage to the papilla

Increased glutaric acid levels are observed in both the liver and the renal papilla and are associated with mitochondrial dysfunction

TRAJECTORIES IN METABOLIC HYPERSPACE FOR TISSUES



Based on ^{1}H MAS NMR spectra from rat renal papilla after administration of BEA

CONSORTIUM ON METABONOMICS IN TOXICOLOGY (COMET)

- Research consortium comprised of 6 (now 5) pharmaceutical companies, work steered by Imperial College over 3 years

- Pfizer Global R&D [Pharmacia Corporation]
  Eli Lilly & Co Bristol-Myers Squibb
  Novo Nordisk A/S F. Hoffmann-la Roche AG

OBJECTIVES

- Generation of a comprehensive database of NMR spectra of rodent biofluids after treatment with various toxicants
- 150 toxins and treatments completed in 3 years (mostly in the rat, some in the mouse)
- Mostly liver and kidney toxins, tested by other organ toxins
- Models of control rat and mouse urine and serum
- Predictive chemometric screening methodologies and novel biomarkers and methods for identifying them

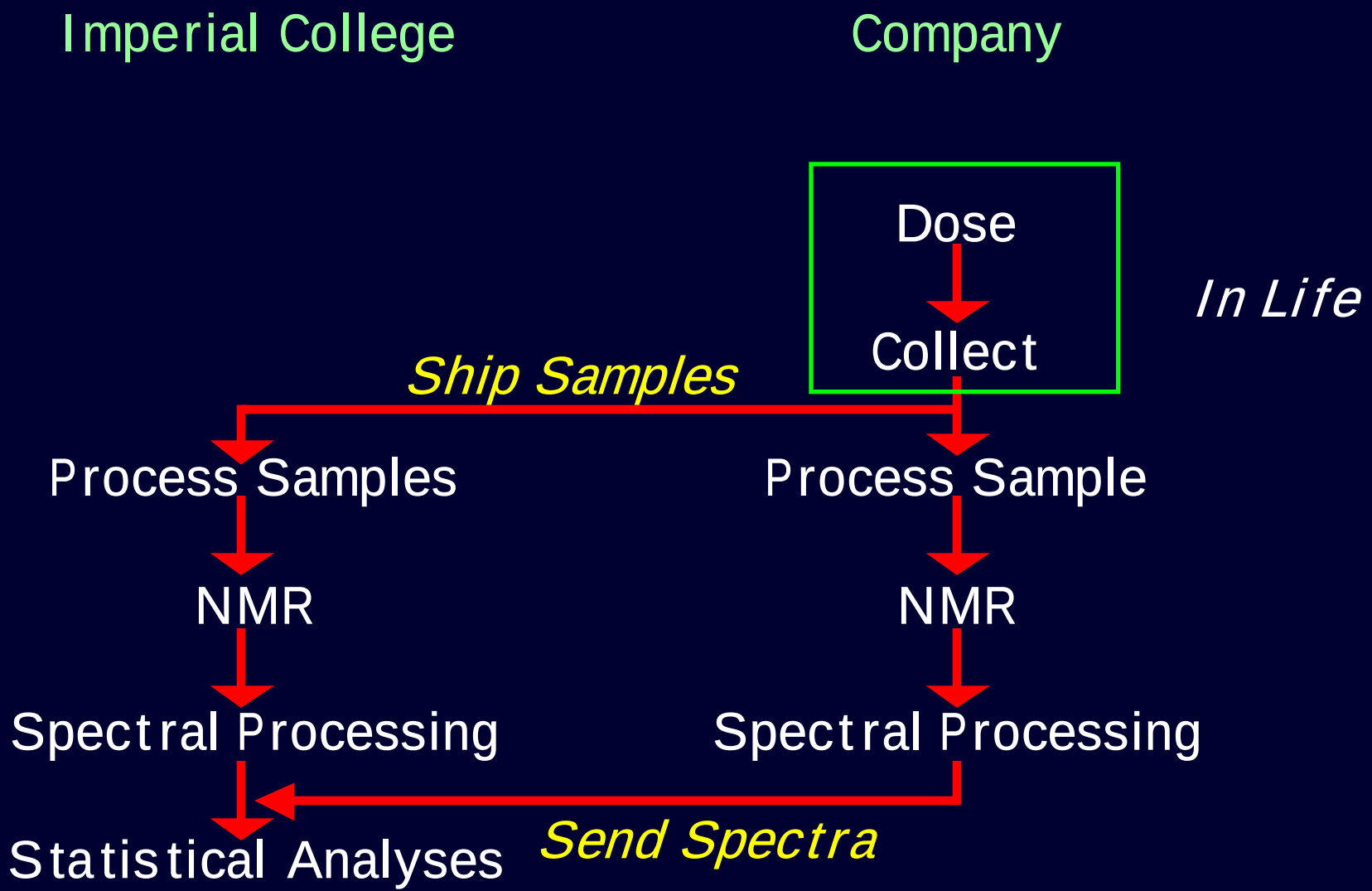
HYDRAZINE "CALIBRATION" STUDY

- Common protocol based on urine and blood serum using hydrazine as a model toxin
- Animal studies and NMR carried out at all sites
- Purpose 1: to determine variability between NMR analyses across sites
- Purpose 2: to determine degree of sample and biological variability across sites

INTERCOMPANY SAMPLE PROVISION COMPARISON

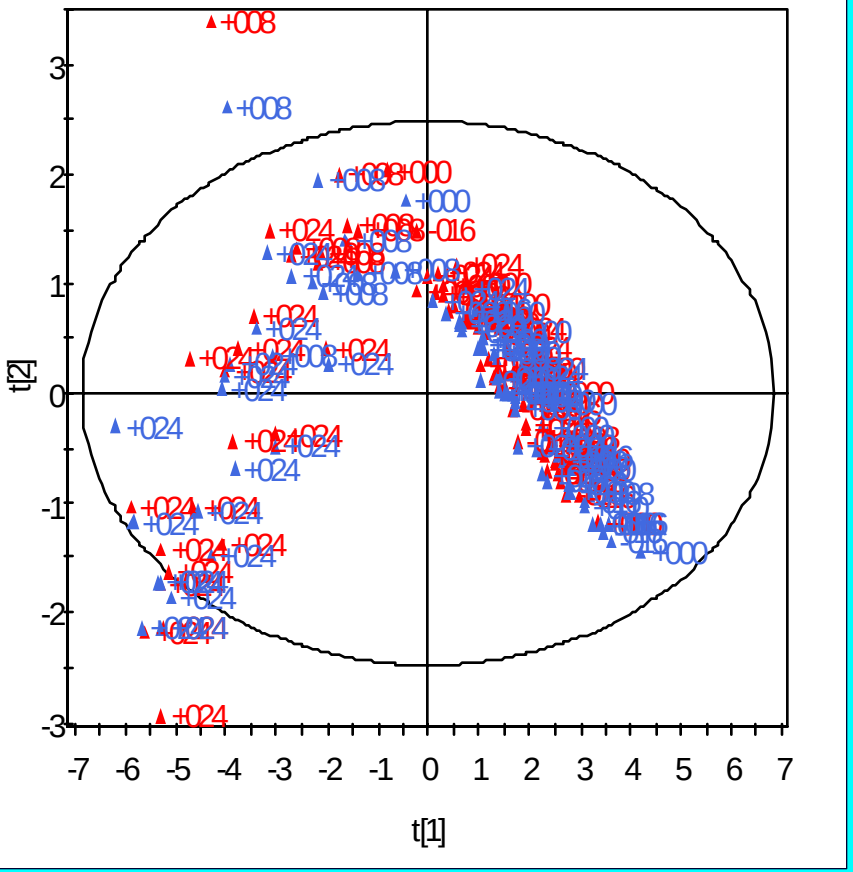
- Study of hydrazine toxicity in the rat
- Blood serum samples at 2 time points – 48 h, 168 h and 3 doses - control, 30 mg/kg, 90 mg/kg
- 4 types of NMR spectra measured – water suppressed, CPMG spin echo, J-resolved, diffusion edited
- Also 7 day urine collection

ANALYTICAL VARIATION TESTING PARADIGM



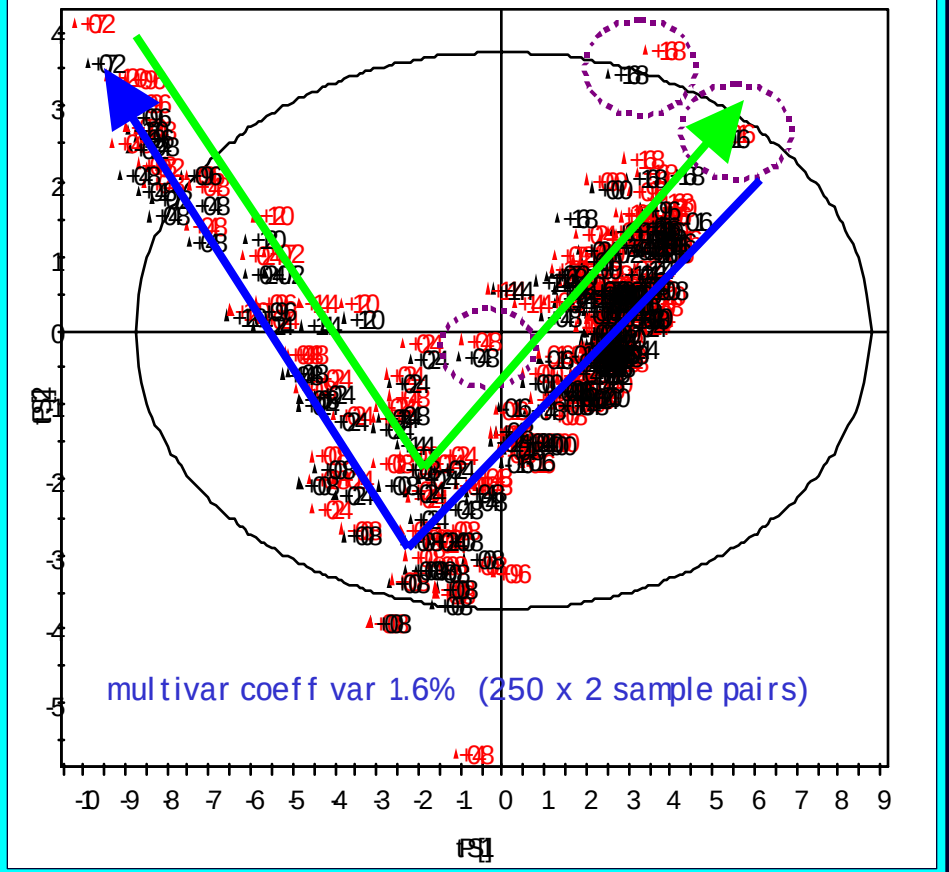
TESTING ANALYTICAL VARIATION

INTER-SITE URINE NMR DATA COLLECTION COMPARISON



Red: Pfizer Blue: IC

Varian and Bruker at 600 MHz

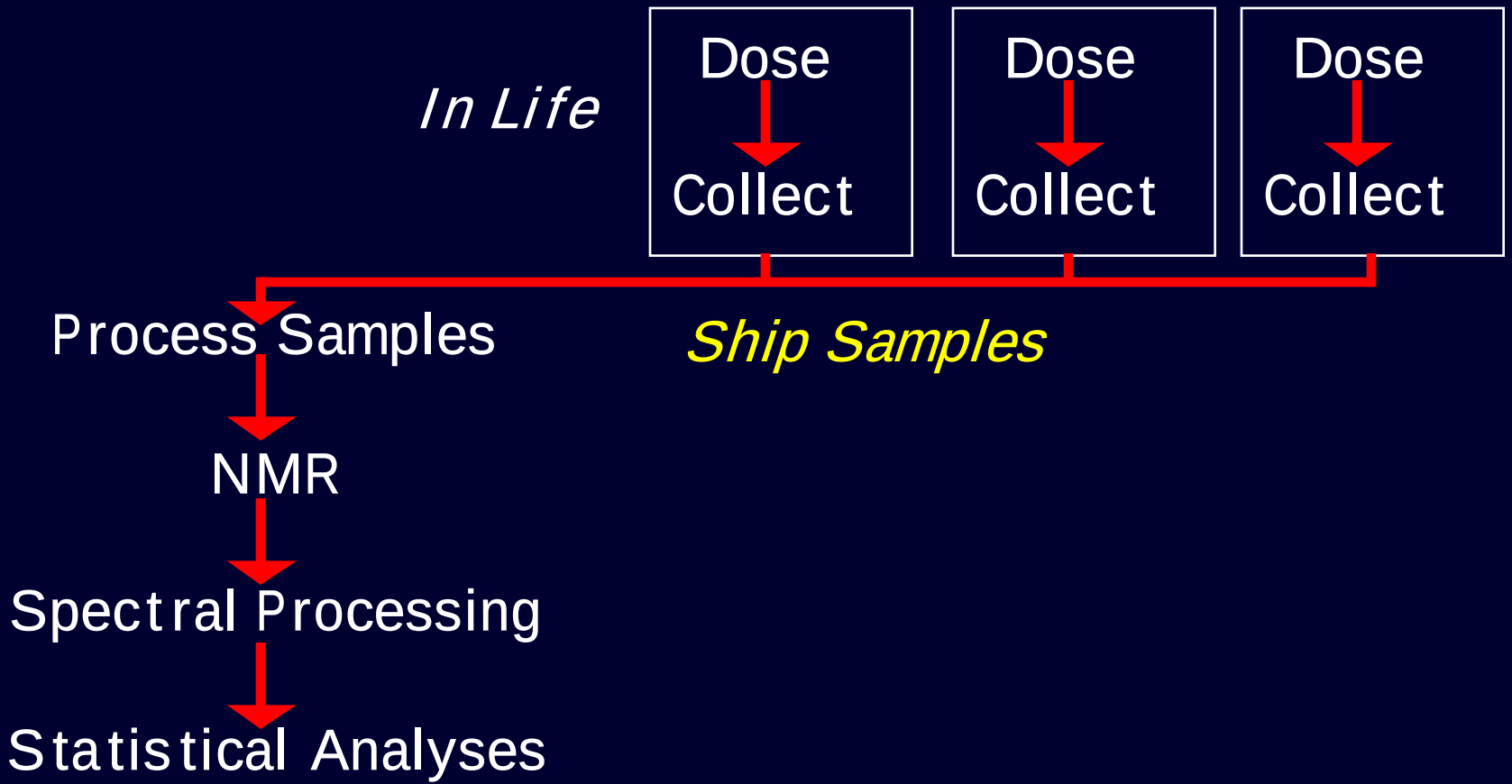


Red: IC Black: Roche

Bruker 500 MHz and 600 MHz

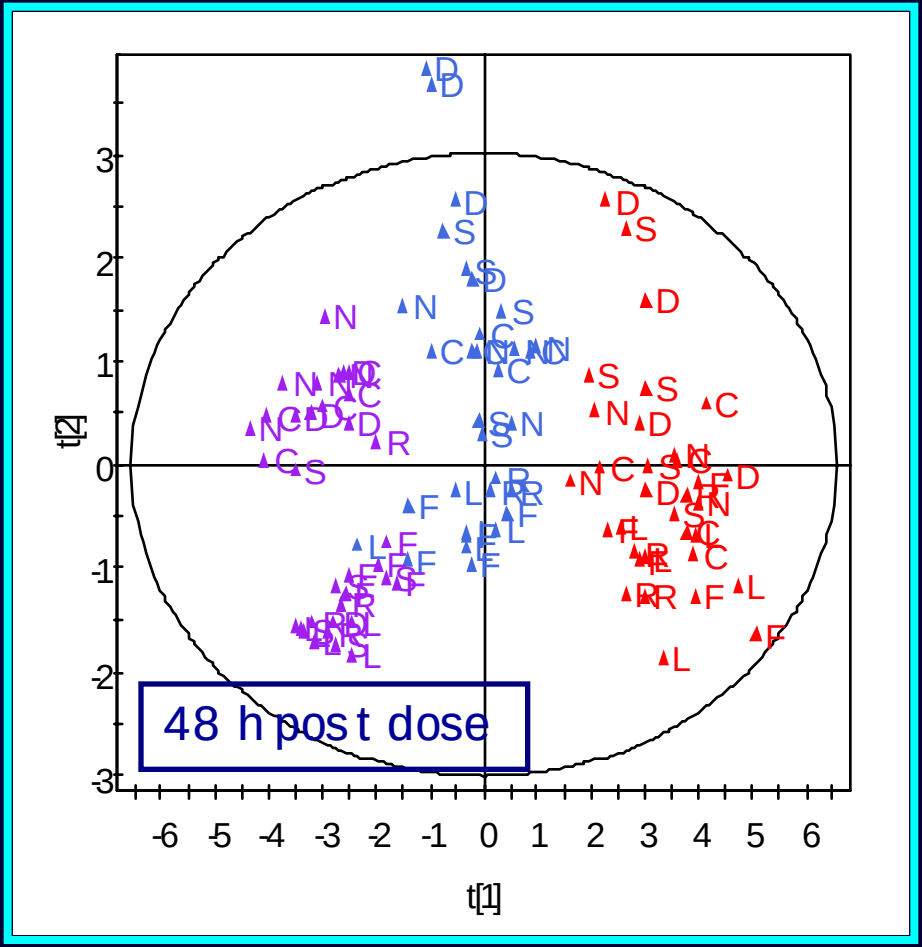
BIOLOGICAL VARIATION TESTING PARADIGM

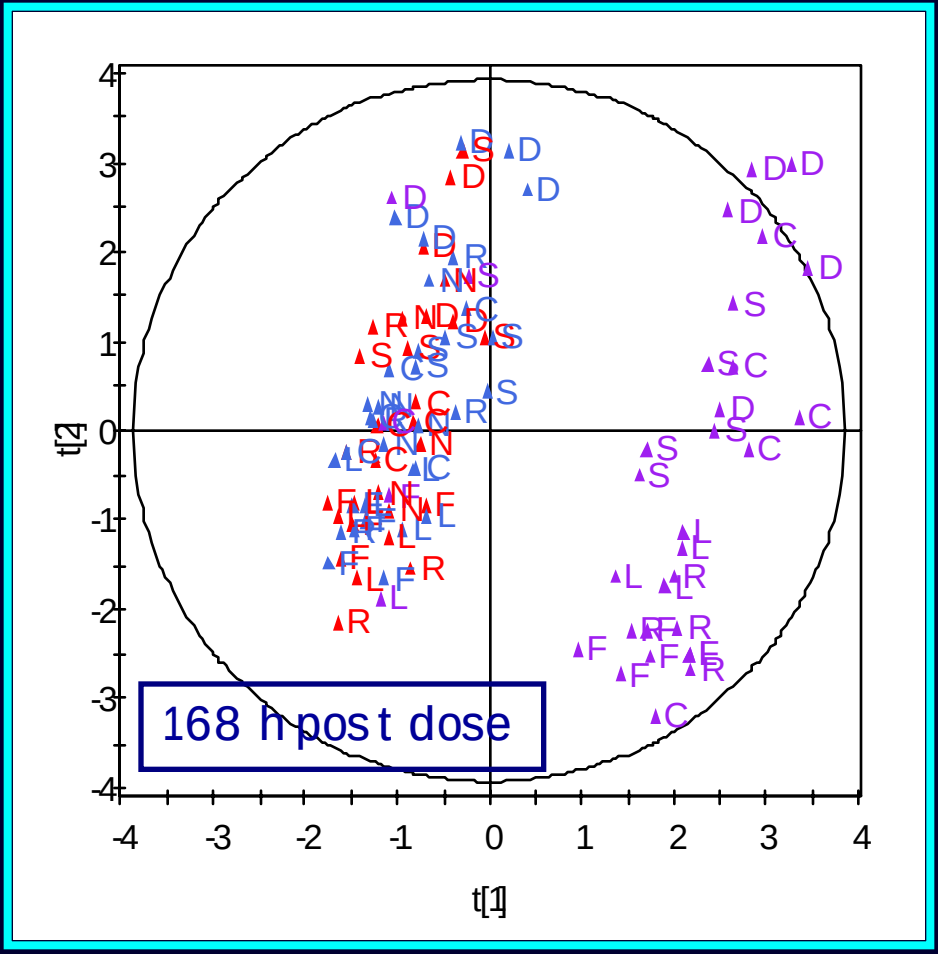
Imperial College Company 1 Company 2 Company 3 etc.



TESTING BIOLOGICAL VARIATION

SAMPLES FROM ALL SITES - SERUM NMR DATA

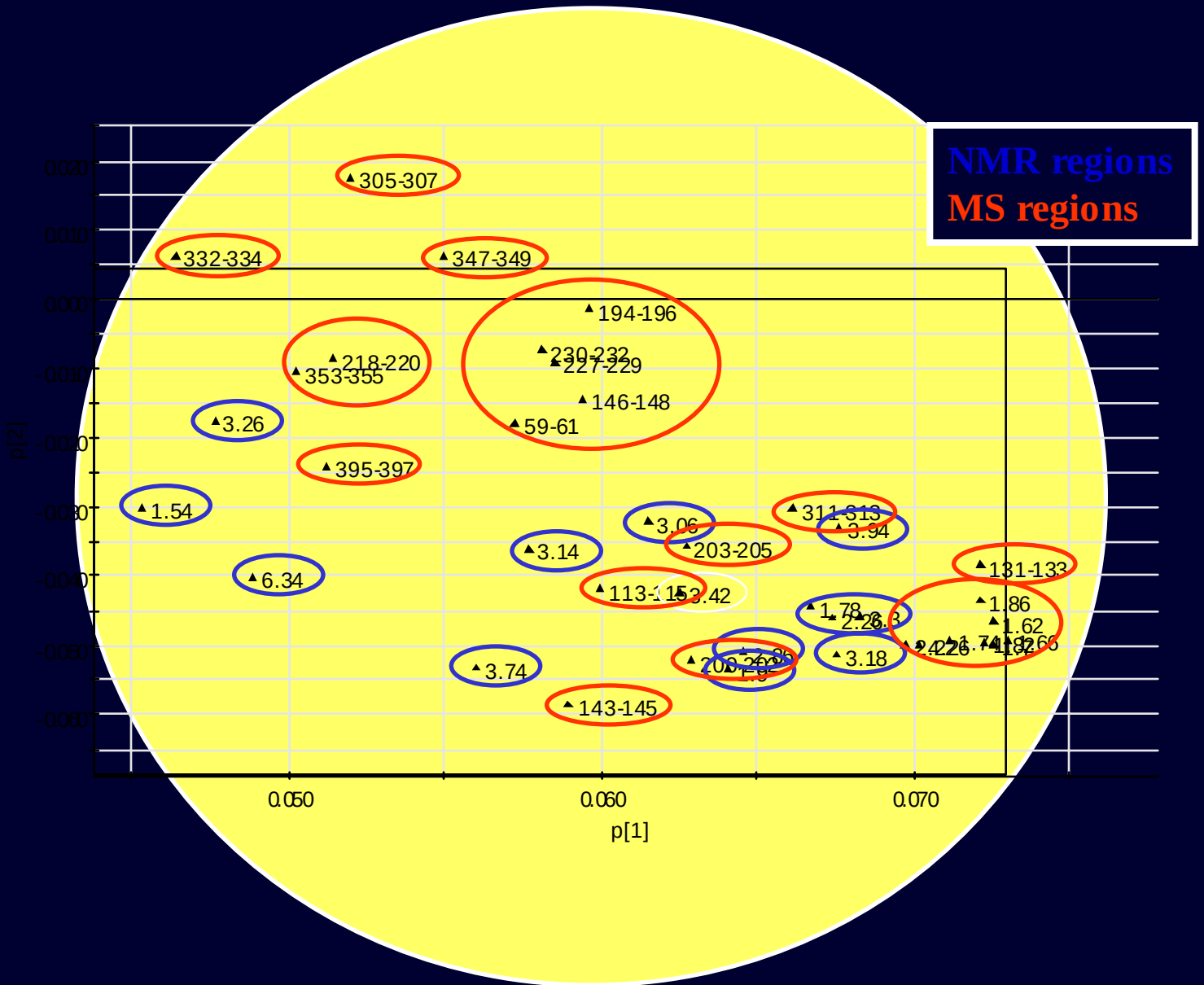




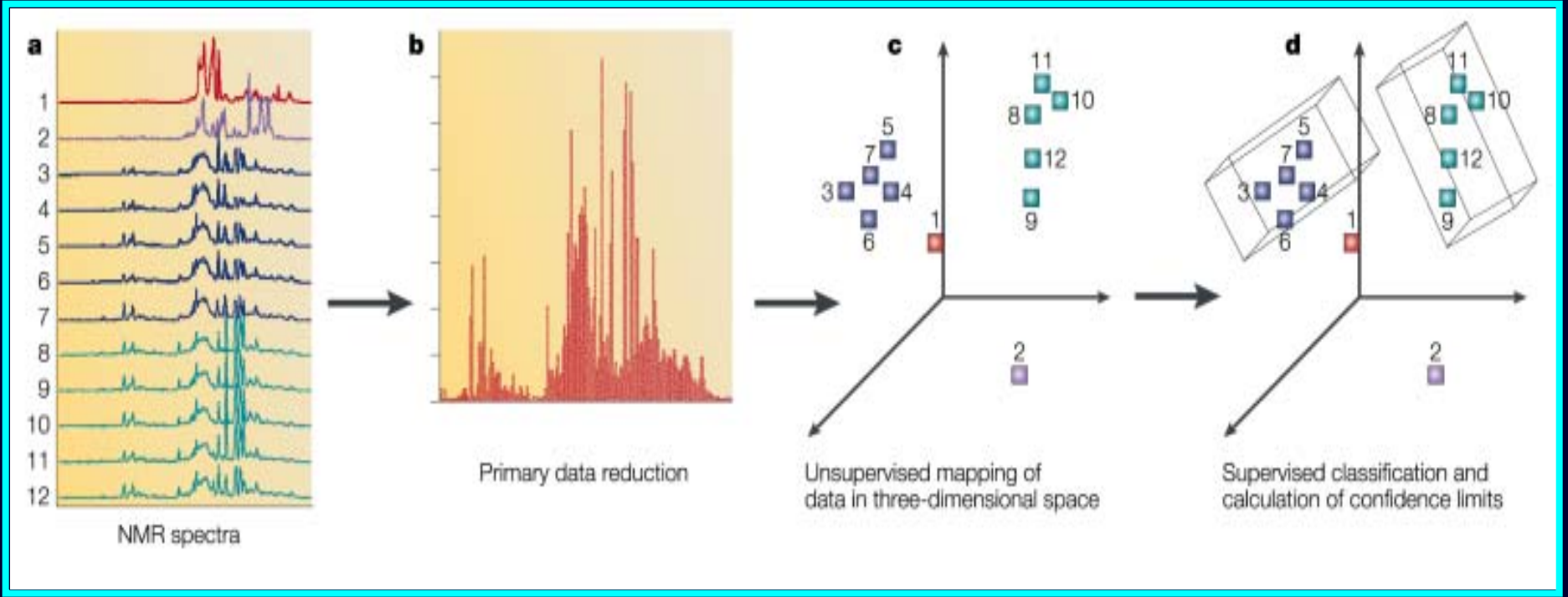
R – Roche, C- Covance, L – Lilly, N – Novo, D – Dupont/BMS, S – Pharmacia, F – Pfizer

Diffusion-weighted spectra 90 mg/kg, 30 mg/kg, controls

COMBINED NMR AND LC-MS PC LOADINGS

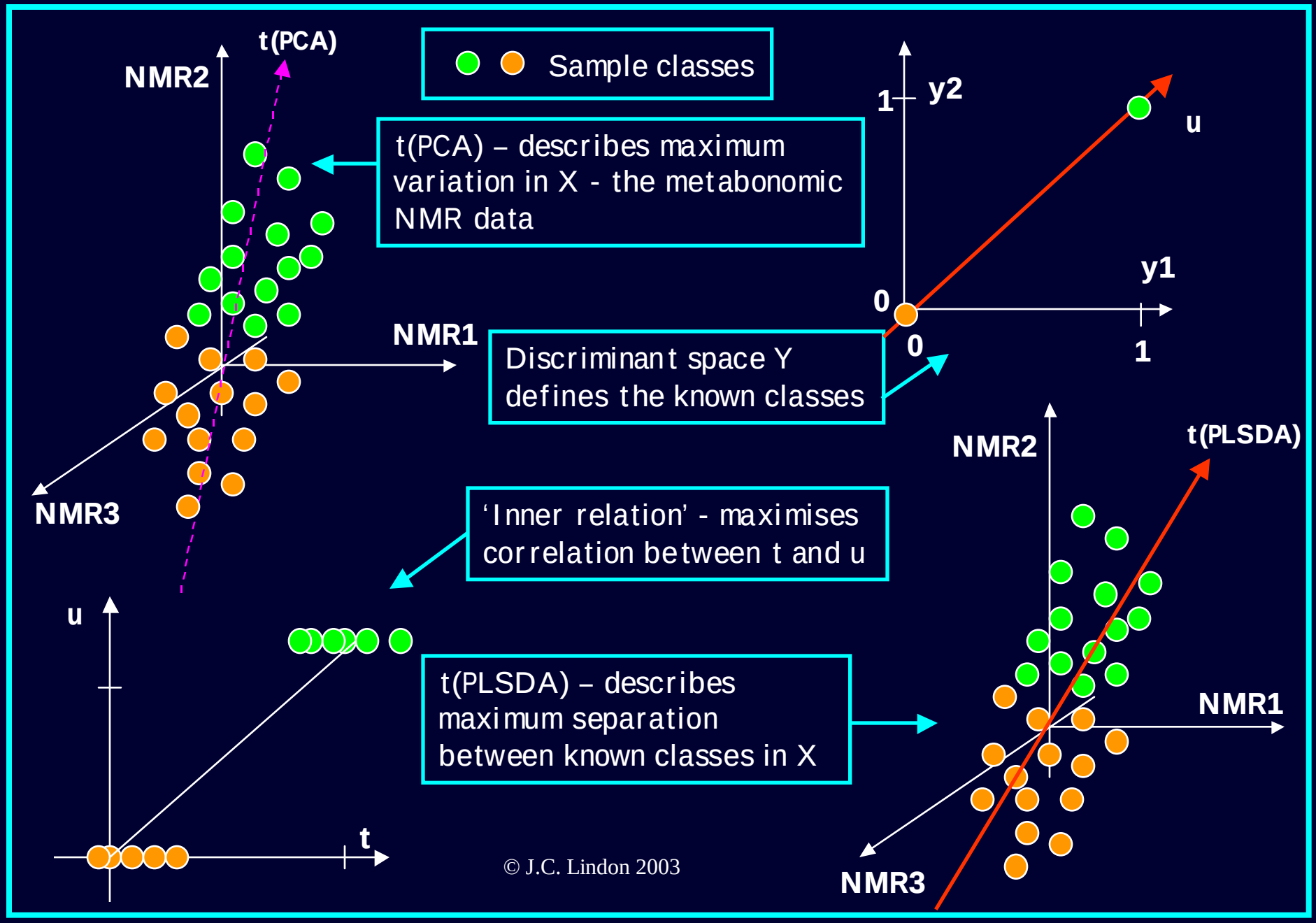


PREDICTIVE MODEL BUILDING

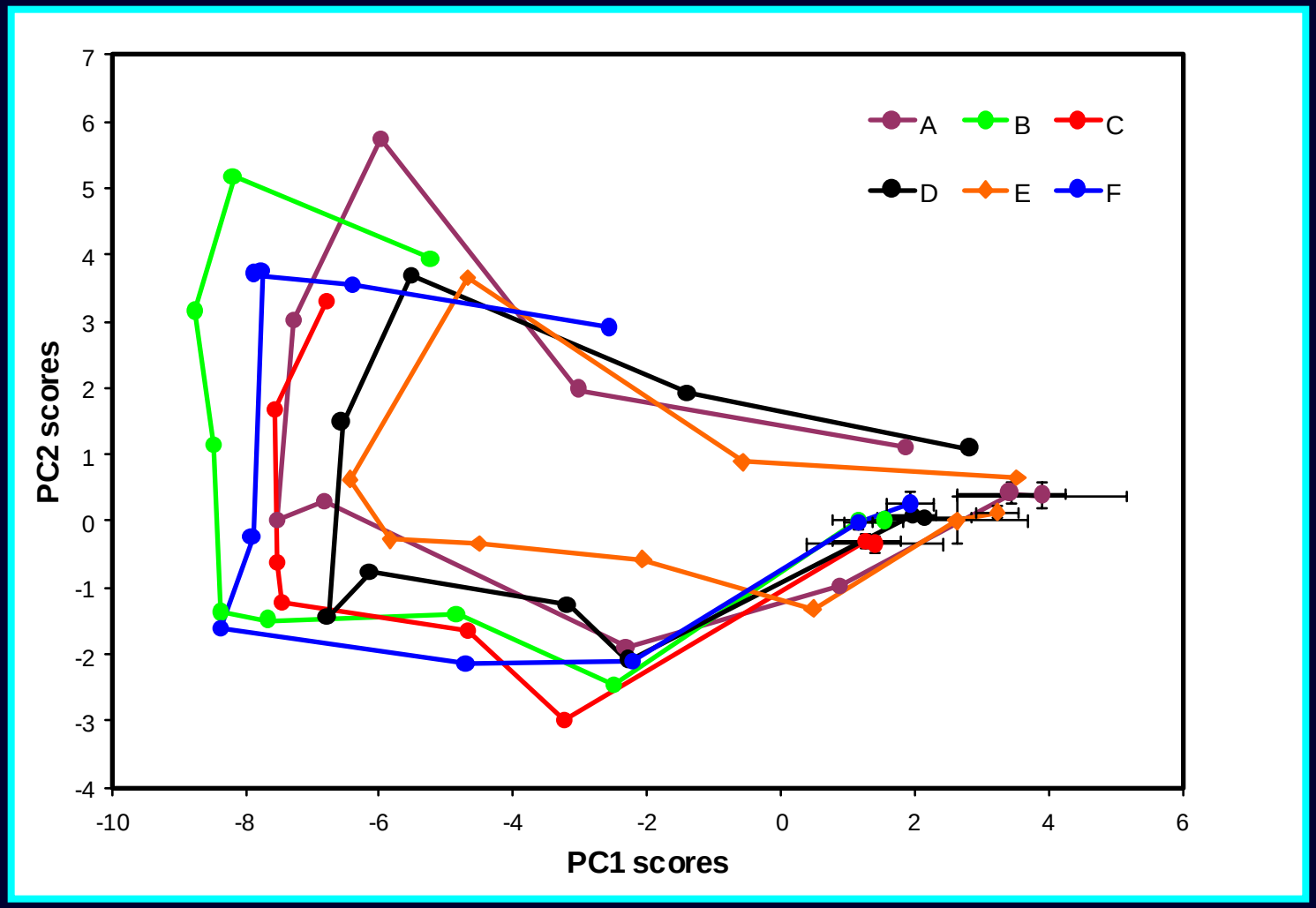


- Build separate statistically-based models for each toxin or disease class (as shown by boxes)
- Test whether new sample (from its NMR spectrum) fits within any model and make class prediction
- Can include confidence limits on classification

PARTIAL LEAST SQUARES DISCRIMINANT ANALYSIS (PLS-DA)

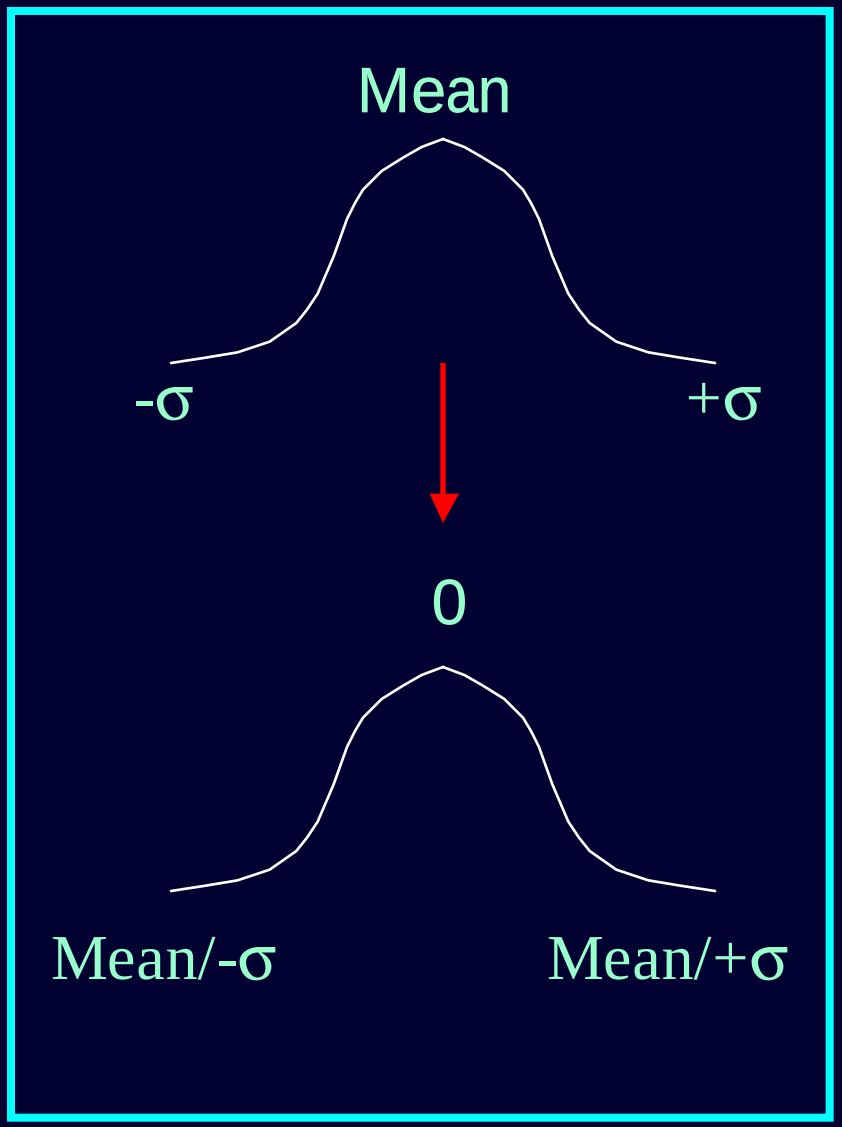


HYDRAZINE INTER-LABORATORY DIFFERENCES AND SIMILARITIES

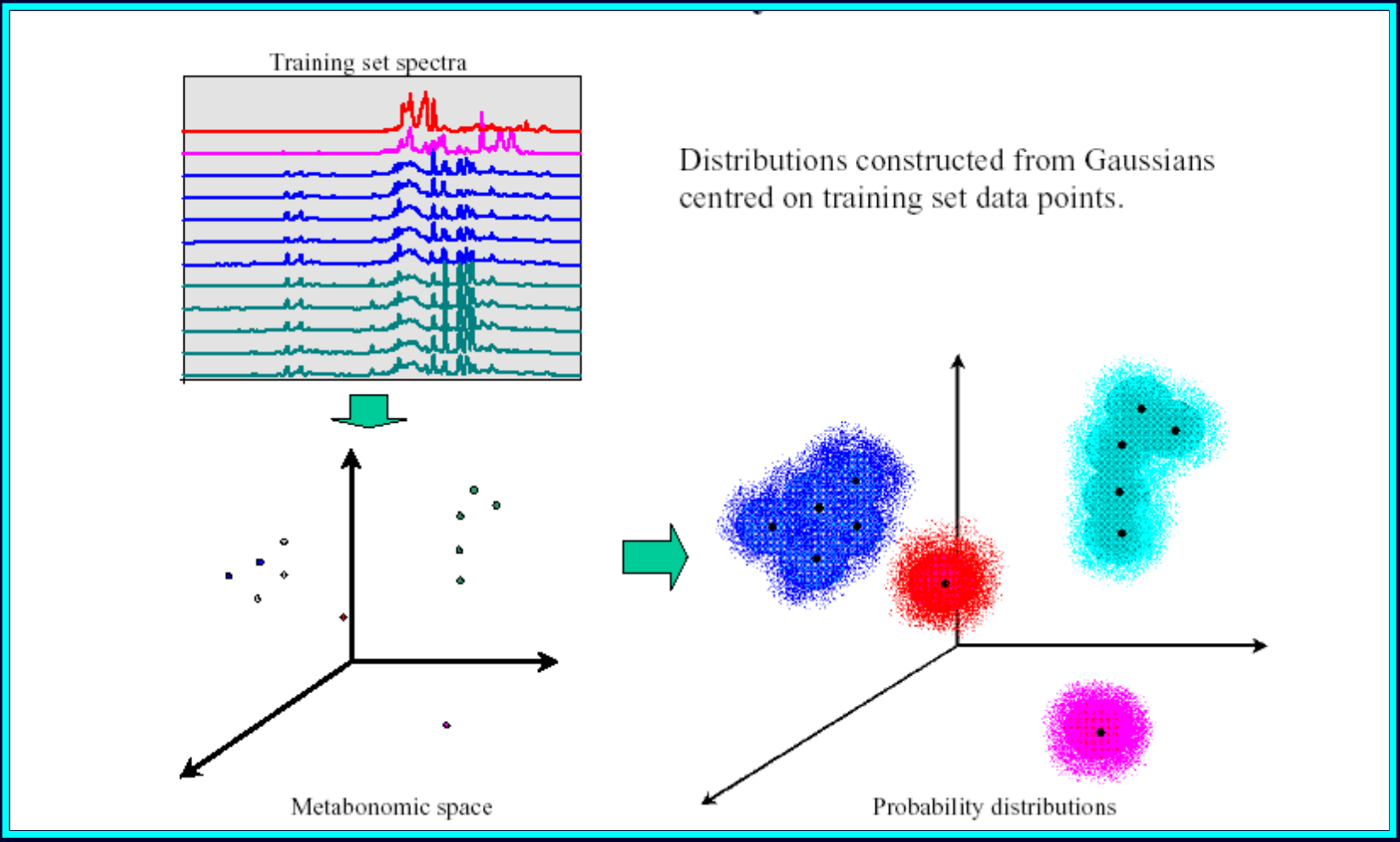


VARIABLE STABILITY (VAST) SCALING

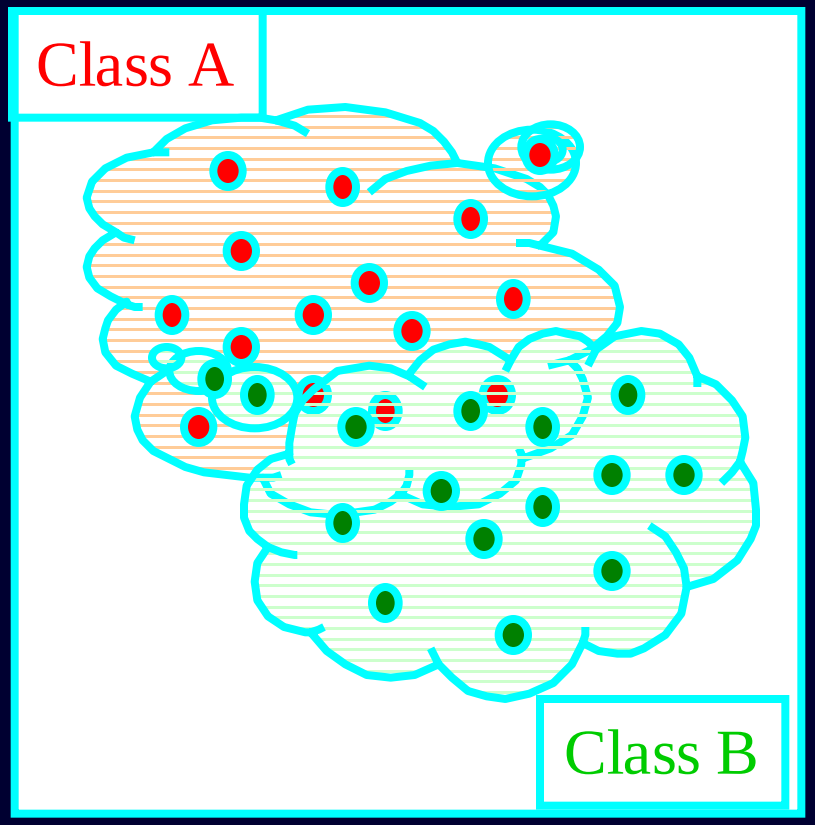
- Calculate stability measure (coefficient of variation) for each variable within each dose group
- Compute the mean of these coefficients to gain a measure of stability within dose groups
- Scale variables using autoscaling, then these coefficients (1/coefficient of variation)



MULTIDIMENSIONAL GAUSSIAN DISTRIBUTIONS



MULTIVARIATE SIMILARITY BETWEEN CLASSES – THE CLOUDS APPROACH



Similarity, ***S*** defined by overlap integral of probability clouds

$$O_{AB} = \int_{\text{all space}} p(\underline{\mathbf{x}}_A) p(\underline{\mathbf{x}}_B) \, dV$$

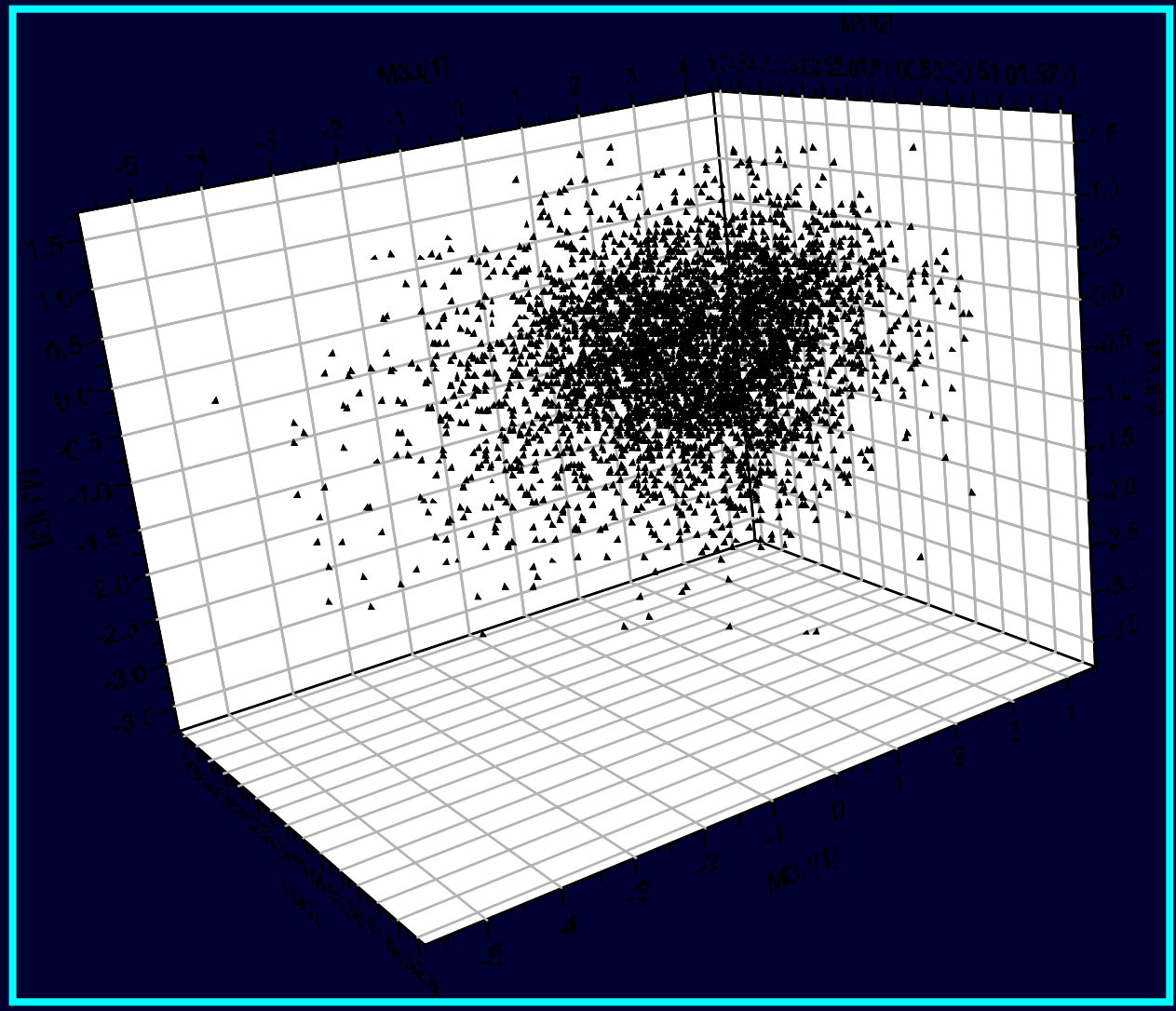
Normalize relative to self overlap:

$$S_{AB} = \frac{O_{AB}}{\sqrt{O_{AA} O_{BB}}}$$

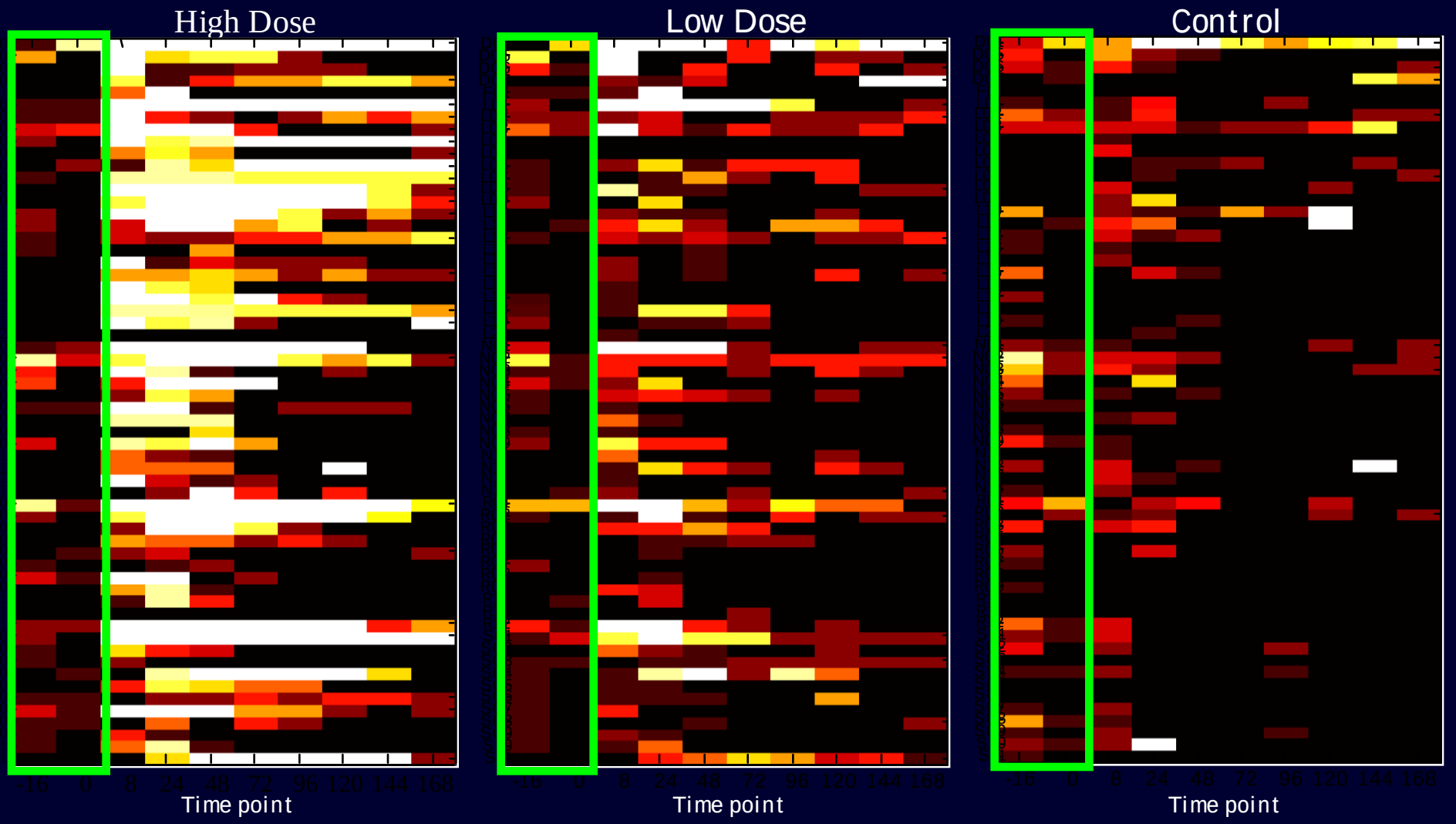
Range: 0 (no overlap, no similarity) → 1 (complete overlap, identity)

MODELLING NORMALITY

PCA of *5,000 600 MHz urine NMR spectra from Control SD rats*

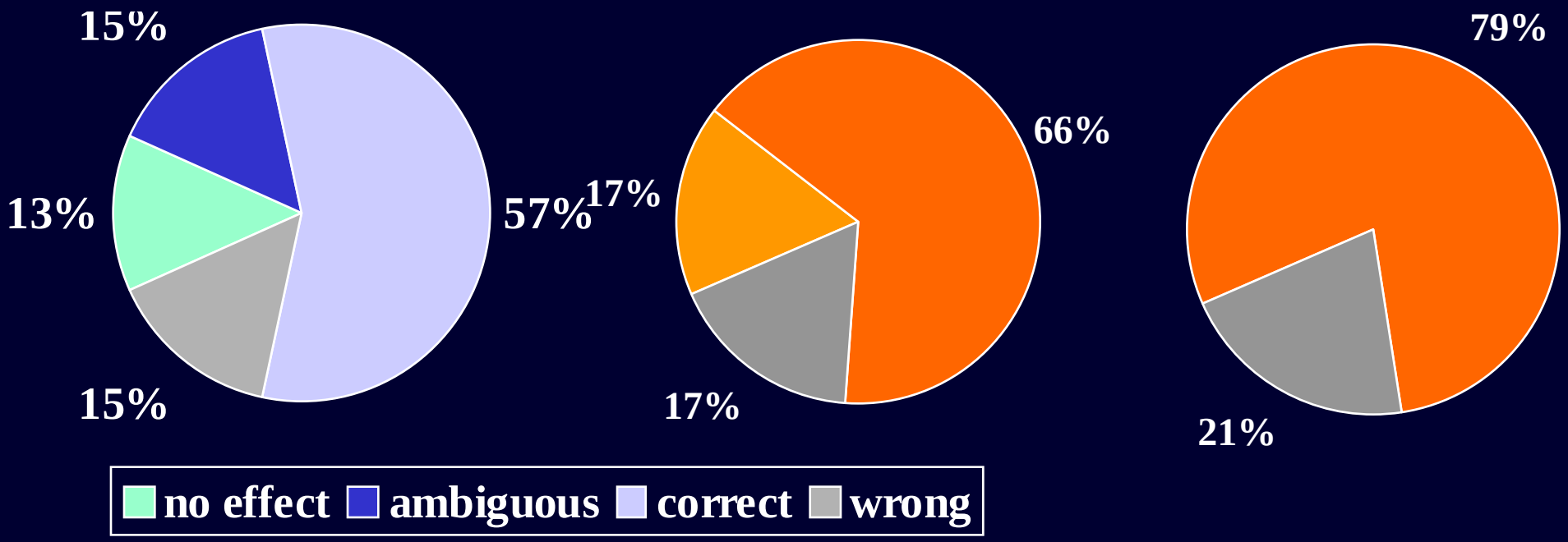


60 TOXICOLOGY STUDIES - DEGREE OF DEVIATION FROM NORMALITY



Key: black = 0% (i.e. control like), white = 100%

PREDICTIONS FROM THE PROTOTYPE EXPERT SYSTEM



CONCLUSIONS

- Metabonomics is a powerful approach for classifying physiological and toxic effects
- Metabonomics can be used to assess age, genetic and environmental effects such as diet, to diagnose disease state, to evaluate genetic modification, to monitor therapeutic efficacy, etc., etc.
- Metabonomics allows an understanding of the time-related events in disease, therapy, altered physiology and drug adverse effects
- Metabonomics can be used effectively to direct and validate proteomic and gene expression data in the real world
- Metabonomics allows the identification of combination biomarkers of physiological and pathological processes

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